

Abstract.—Microsatellite DNA variation at six microsatellite loci (Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108) was examined in approximately 900 sockeye salmon, *Oncorhynchus nerka*, collected between 1987 and 1995 from three stocks on the west coast of Vancouver Island, British Columbia, Canada. Variation in allele frequencies among stocks was, on average, about 12 times greater than temporal variation within stocks. Individual locus F_{ST} estimates ranged from 0.013 to 0.107 among stocks, with an overall value of 0.056. Analysis of simulated mixed-stock samples indicated that data from four to six of the microsatellite loci surveyed would enable relatively accurate and precise estimates of stock composition for mixtures composed of fish from the three stocks. Application of the mixture analysis to 1100 fish sampled in Barkley Sound and Alberni Inlet fisheries during 1997 indicated that sockeye salmon from Great Central Lake constituted about 70% of the commercial catch. The later time of return of sockeye salmon from Henderson Lake than of those from Great Central or Sproat Lake as previously indicated by analysis of parasite frequencies was confirmed in the 1997 fishery sampling. Stock composition of catches varied among gears, presumably owing to gear selectivity.

Microsatellite DNA variation and estimation of stock composition of sockeye salmon, *Oncorhynchus nerka*, in Barkley Sound, British Columbia

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In the sockeye salmon (*Oncorhynchus nerka*) fishery in Barkley Sound on the west coast of Vancouver Island, three stocks (Sproat Lake, Great Central Lake, and Henderson Lake) account for all of the catch in the mixed-stock fishery (Hyatt and Steer, 1987) (Fig. 1). These stocks have been exploited for over 100 years, but the area of the fishery has changed. The present fishery is conducted over a wide area in Barkley Sound. Lake fertilization has been used to increase production of Barkley Sound sockeye salmon (LeBasseur et al., 1978; Hyatt and Stockner, 1985). Of the lakes sampled in our study, Great Central Lake has been fertilized most extensively, with annual applications of fertilizer between 1970 and 1973, and from 1977 to the present. Sproat Lake was fertilized between 1985 and 1987, and Henderson Lake has been fertilized from 1976 to the present.

Assessment of the effects of fertilization on the productivity of Great Central and Henderson lakes required accurate and reasonably precise estimates of stock composition in the Barkley Sound sockeye salmon catch. The frequency of occurrence of two myxosporean parasites, *Myxo-*

bolus arcticus in the brain and *Heneguya salmonicola* in the muscle, differed substantially among sockeye salmon in the three lakes during 1977–84 (Quinn et al., 1987), and these differences in prevalence were used to provide estimates of stock composition in the fishery until 1984 (Steer et al., 1986, 1988). Sockeye salmon from Sproat Lake and Great Central Lake accounted for 95% of the catch from 1980 to 1984 (Hyatt and Steer, 1987). In the 1990s, it became apparent that the frequency of occurrence of the two parasites had changed in Great Central Lake sockeye salmon (Beacham et al., 1998), and fishery managers no longer considered estimates of stock composition derived from parasites to be reliable for management decisions. The timing of the change in parasite frequency of occurrence between 1984 and the 1990s was unknown, rendering post-1984 estimates of stock composition and associated estimates of individual lake productivity uncertain. It became imperative to develop a reliable alternative method of stock identification that could be applied to fishery samples for accurate estimation of both catch and productivity by stock.

A preliminary survey of DNA variation at microsatellite loci indicated that there was some differentiation among the Barkley Sound sockeye salmon stocks (Nelson et al., 1998). Evaluation of alternative methods of stock identification indicated that mixture analysis based on microsatellite allele frequencies would likely provide reliable estimates of stock composition (Beacham et al., 1998). In the present study, we expanded the analysis of variation at microsatellite loci of Barkley Sound sockeye salmon to six polymorphic loci, examined the differentiation among and within stocks at each locus, evaluated the precision of data and accuracy of stock composition estimates for a range of mixture sample sizes based on data from three to six loci, and finally used the microsatellite variation to estimate stock compositions from 1997 fishery samples.

Materials and methods

Collection of DNA samples and amplification by PCR

Scales were collected from sockeye salmon returning to spawn in the Sproat Lake and Great Central Lake drainages in 1987, 1990, and 1992. Scales were collected from Henderson Lake sockeye salmon in 1988 and 1993, and liver samples preserved in 95% ethanol were collected in 1995. Scales or operculum punches were collected from sockeye salmon sampled in fisheries in 1997. DNA was extracted from scales as outlined by Nelson et al. (1998). For the operculum or liver samples, approximately 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 mL of 5% chelex in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA pH 8.0, 0.10 mg/mL proteinase K, and 0.1% SDS) and incubated for 15 min at 50°C, and then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at -20°C. About 1 mL of this extract was required for each amplification of the sample by the polymerase chain reaction (PCR).

Loci amplified by PCR were the dinucleotide repeats Omy77 and Ots3 and the tetranucleotide repeats Ots100, Ots103, Ots107, and Ots108 (Table 1). For all primer sets used in this study, PCR was conducted in 25- μ L reactions containing 12 pmol (0.48 μ M) of each primer, 80 μ M of each nucleotide, 20 mM Tris-pH

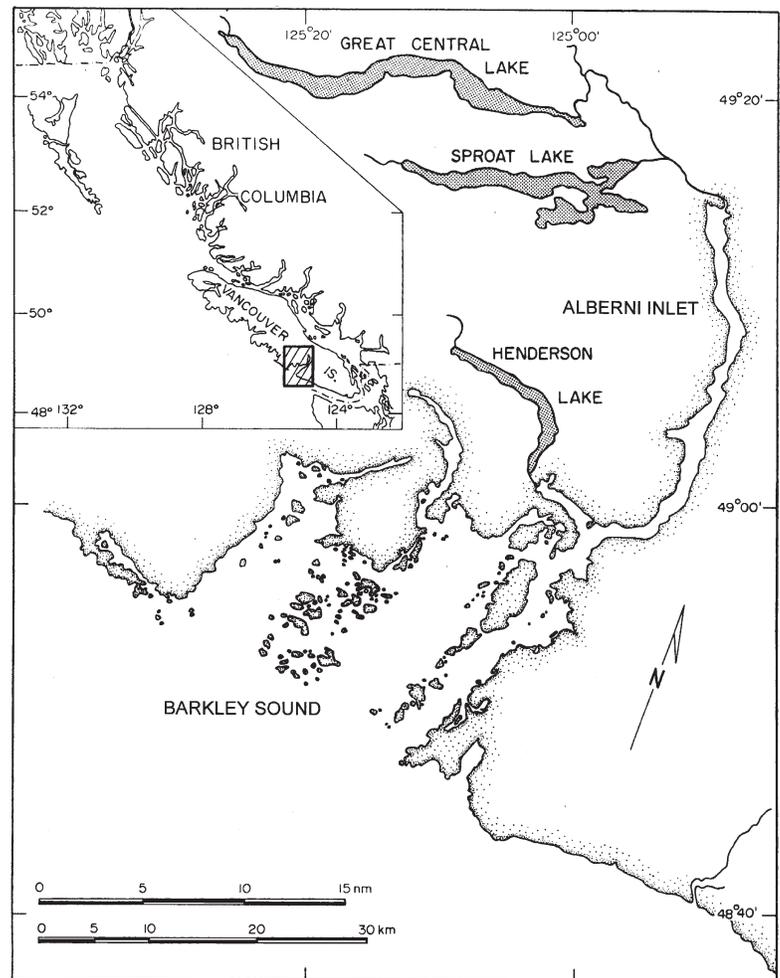


Figure 1

Location of Barkley Sound on Vancouver Island. Sockeye salmon are produced in Great Central Lake and Sproat Lake, both part of the Somass River drainage, as well in Henderson Lake.

8.8, 2 mM MgSO₄, 10 mM KCl, 0.1% Triton X-100, 10 mM (NH₄)₂SO₄, and 0.1 mg/mL of nuclease-free bovine serum albumin. Each PCR reaction was preceded by an initial denaturation step of three min at 94°C. All cycle extension (30 cycles for all loci except Ots108 which was 35 cycles) steps were for 60 sec at 72°C and all cycle denaturation steps were for 20 sec at 94°C. PCR of Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108 was accomplished with annealing temperatures of 48°C, 50°C, 57°C, 55°C, 48°C, and 46°C, respectively. Annealing times were 30 sec for Omy77 and Ots100, and 60 sec for the other loci.

Gel electrophoresis and band analysis

PCR products were size fractionated on 16 cm × 17 cm nondenaturing polyacrylamide gels and visualized by

staining with 0.5 mg/mL ethidium bromide in water and ultraviolet light illumination. Nelson et al. (1998) provide a complete description of gel electrophoretic conditions. All gels were run for 14–18 h at 65–70 V, using 8% acrylamide for analysis of Ots100 and Ots103, and 10% acrylamide for analysis of Omy77, Ots3, Ots107 and Ots108. Twenty-nine lanes per gel were loaded. One outside lane contained a one-kb ladder (Gibco BRL), three lanes contained a 20-bp ladder (Gensura Labs Inc., Del Mar, CA) evenly spaced across the gel, one lane contained a standard fish to determine precision of estimation of allele size, and 24 lanes contained an individual fish for analysis.

Gels were scanned at a 1024 × 1024 pixel density with a Kodak charge coupled device (CCD) camera with low-light capability and a yellow filter. Images were analyzed by using BioImage Whole Band software (Genomic Solutions Inc., 1995), where the size of the amplified microsatellite alleles were reported to the nearest base pair (bp) based upon the molecular size grid created with the 20-bp markers.

Because some uncertainty occurred in estimation of allele size from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill et al., 1990). Peaks in the allele frequencies used to identify main alleles and bin widths generally corresponding to a repeat unit were set so that the main allele was located in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analyzed for each locus.

Data analysis

Annual variation in allele frequencies within populations was tested with GENEPOP version 3.1 with the Markov-

Chain approach by using χ^2 probability values (Raymond and Rousset, 1995). The dememorization number was set at 1000, and 50 batches were run for each test with 1000 iterations/batch (Raymond and Rousset, 1995). Each stock at each locus was tested for departure from Hardy-Weinberg equilibrium by using GENEPOP. Gametic linkage disequilibrium between loci in each population was also evaluated with GENEPOP. Tests of genetic differentiation with three pairwise comparisons among the populations were also conducted with GENEPOP with the Markov-Chain approach by using χ^2 probability values. Critical significance levels for simultaneous tests were evaluated by using sequential Bonferroni adjustment (Rice, 1989). F_{ST} estimates for each locus were calculated with GENEPOP, and the standard deviation of the estimate for an individual locus was determined with FSTAT (Goudet, 1995) by jackknifing over stocks and for all loci combined by bootstrapping over loci. Estimation of variance components of stock differences and annual variation within stocks was determined with BIOSYS (Swofford and Selander, 1981). Principal components of nine (three annual samples multiplied by three stocks) composite arrays of allele frequencies for six loci were calculated with the PRINCOMP procedure in SAS (SAS, 1989).

Estimation of stock composition

The effectiveness of using variation at microsatellite loci for the practical assessment of stock composition in mixed-stock fisheries of Barkley Sound was evaluated from the stand points of precision of stock composition data and accuracy of stock composition estimates in simulated fishery samples. Although only three stocks could contribute to the fishery samples, we wished to determine the sample size required to detect accurately the relatively small proportion of Henderson Lake sockeye salmon that were expected to be present in most fishery samples. In addition, we wished to examine the effect of the number of loci used in the estimation of stock composition. The simulated mixtures were composed of 30% Sproat Lake fish, 60% Great Central Lake fish, and 10% Henderson Lake fish because these proportions are the approximate long-term mean of the Barkley Sound fishery.

Allele frequencies were determined for each locus in each stock, and the model of Fournier et al. (1984) was used to estimate stock composition by the condi-

Table 1

Primer sequences for the microsatellite loci analyzed in the study.

Locus	Sequence (5'–3')	Source
Omy77	F: CGT TCT CTA CTG AGT CAT R: GGG TCT TTAAGG CTT CAC TGC A	Morris et al. (1996)
Ots3	F: CAC ACT CTT TCA GGA G R: AGA ATC ACA ATG GAA G	Banks et al. (1999)
Ots100	F: TGA ACA TGA GCT GTG TGA G R: ACG GAC GTG CCA GTG AG	Nelson et al. (1998)
Ots103	F: AGG CTC TGG GTC CGT G R: TGA TAT GGT GTG ATA GCT GG	Beacham et al. (1998)
Ots107	F: ACA GAC CAG ACC TCA ACA R: ATA GAG ACC TGA ATC GGT A	Nelson and Beacham (1999)
Ots108	F: TCT GTT TAT CTT TCT ATT A R: AAG GAG AGA CAG AGG G	Nelson and Beacham (1999)

tional maximum likelihood method. Baseline genotypic frequencies for each of the three stocks were calculated from the observed allele frequencies under the assumption of Hardy-Weinberg equilibrium. Each baseline stock was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture. Hypothetical fishery samples of 100–300 fish with fixed stock composition were generated by randomly resampling with replacement the baseline stocks, and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined, and the whole process was repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates.

Fishery samples

In 1997, samples were collected from three commercial gillnet fishery openings in Barkley Sound, a gillnet test fishery, a purse-seine test fishery, the recreational fishery, and an aboriginal fishery. The commercial gillnet fishery was conducted primarily in Barkley Sound, with gillnet mesh sizes ranging from 114 mm (4.5 inches) to 133 mm (5.25 inches). The gillnet test fishery was conducted farther inland at the head of Barkley Sound and at the mouth of Alberni Inlet with a gill net 110 m (60 fathoms) in length and 180 meshes deep, and having a mesh size of 114 mm. Samples from the purse-seine fishery, the recreational fishery, and the aboriginal fishery were derived entirely from Alberni Inlet. The recreational fishery was conducted near the head of Alberni Inlet and the aboriginal fishery, conducted at the head of Alberni Inlet and in the Somass River, was the most terminal fishery. Estimated stock contributions to each sample were determined as a point estimate from all the fish in the sample, and standard deviations of the estimates were derived from bootstrap resampling of both the baseline stocks and the mixture.

Results

Precision of estimation of allele size

Standard deviations of the estimated allele sizes for the heterozygous standard fish analyzed at each locus

Table 2

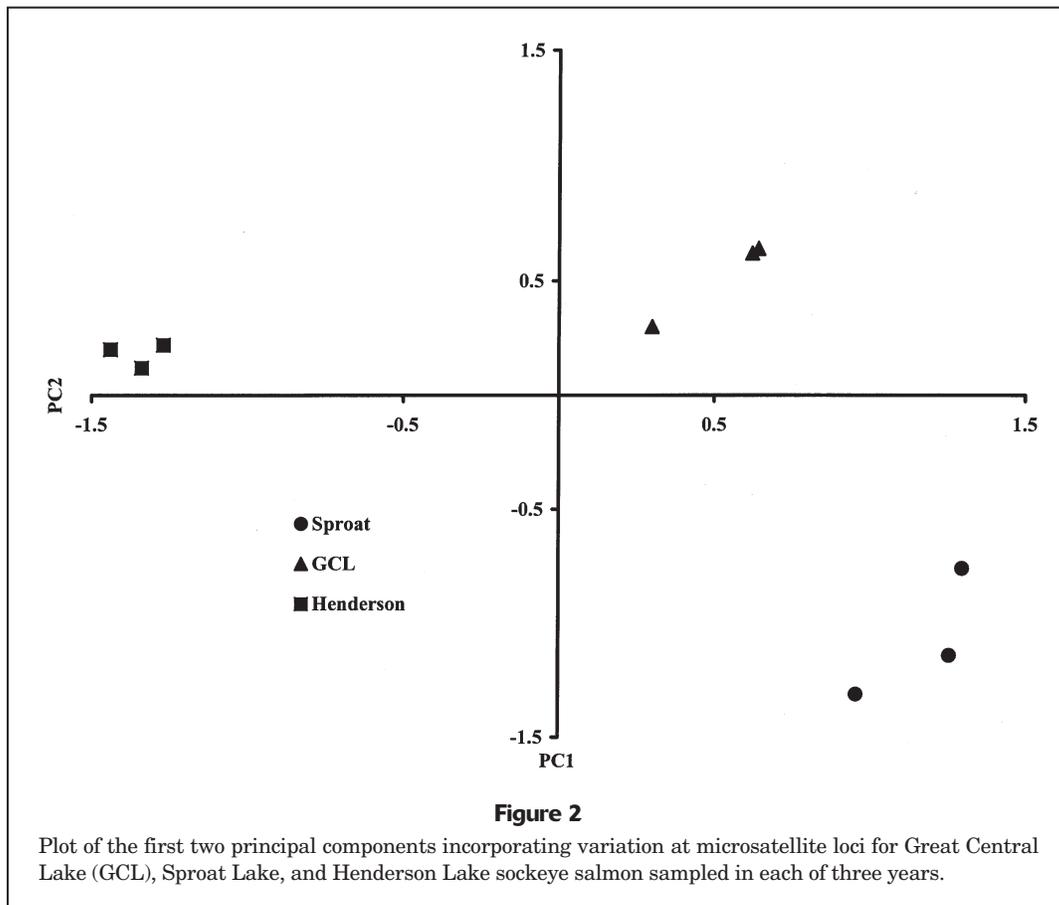
Precision of estimates of allele size (in basepairs) at each microsatellite locus for standard fish run only once per electrophoretic gel. *n* is the number of gels on which allele sizes for a standard fish were estimated. Standard deviation is given in parentheses.

Locus	<i>n</i>	Allele size	Range	Allele size	Range
Ots3	15	74.1 (0.35)	74–75	93.1 (0.35)	93–94
Omy77	28	94.9 (0.63)	94–96	110.3 (0.53)	109–111
	24	100.4 (0.53)	100–101	116.0 (0.62)	115–117
	8	104.0 (0.00)	104–104	116.1 (0.64)	115–117
Ots107	46	109.7 (0.55)	109–111	117.7 (0.48)	117–118
Ots108	12	112.1 (0.67)	111–113	184.6 (0.51)	184–185
Ots100	8	158.3 (0.71)	157–159	184.4 (1.30)	183–186
	26	164.5 (0.71)	163–166	181.6 (0.64)	180–183
	11	158.4 (0.50)	158–159	196.5 (0.52)	196–197
Ots103	33	175.1 (0.60)	174–176	213.4 (1.00)	211–215

ranged from 0.00 to 1.30 and tended to increase with allele size (Table 2). For both alleles at Ots3, 100% of the estimated sizes for each allele spanned a 2-bp interval. For alleles <110 bp at Omy77, 93% (56/60) of the estimated sizes of the allele were in a 2-bp interval, as were 90% of the estimated sizes of alleles between 110 and 120 bp. Estimated sizes of alleles of the standard fish that were analyzed at the other loci were all estimated within a 4-bp interval for alleles <200 bp, with 85% of the estimated sizes of the larger allele (213 bp) at Ots103 within a 4-bp interval.

Variation within stocks

All six microsatellite loci examined were polymorphic for all three stocks. Observed heterozygosity of the loci examined over all stocks was as follows: Omy77 0.70 (stock range 0.61–0.80), Ots3 0.67 (0.64–0.70), Ots100 0.75 (0.69–0.79), Ots103 0.83 (0.81–0.86), Ots107 0.28 (0.17–0.40), and Ots108 0.85 (0.80–0.89). Significant departures (correction for three tests per locus, $\alpha=0.0167$) from the expected Hardy-Weinberg distribution of genotypic frequencies were observed at the Omy77 locus in all stocks, owing, in the case of Sproat and Henderson lakes, to a deficiency of heterozygotes. A similar significant heterozygote deficiency was also detected at Ots108 in Sproat Lake sockeye salmon. Significant annual variation (correction for six tests per stock, $\alpha=0.0083$) in allele frequencies was observed at Omy77 in Sproat Lake and Henderson Lake sockeye salmon, and at Ots108 in Henderson Lake sockeye salmon. No significant linkage disequilibrium between any pair of loci in any stock was observed.



Variation among stocks

The three sockeye salmon stocks in Barkley Sound were genetically distinct at all six loci examined. All pairwise tests of allele frequencies among stocks were highly significant at all loci ($P < 0.001$). At Omy77, the frequency of the Omy77⁹⁴ allele ranged from 0.005 in Henderson Lake sockeye salmon to 0.449 in Sproat Lake fish, and the frequency of Omy77¹⁰⁴ ranged from 0.216 in Sproat Lake fish to 0.546 in Henderson Lake fish (Table 3). Substantial differentiation in allelic frequencies among stocks was observed at Ots3. For example, the frequency of Ots3⁸⁸ in Henderson Lake sockeye salmon was 0.114, whereas in Sproat Lake fish it was 0.543. Similarly, the frequency of Ots3⁹⁹ was 0.059 in Sproat Lake fish and 0.243 in Henderson Lake fish. At Ots100, the frequency of Ots100¹⁵⁸ ranged from 0.256 in Sproat Lake sockeye salmon to 0.504 in Henderson Lake fish (Table 3). Although the three stocks were distinct at Ots103, the allele frequency variation was less marked at that locus. At Ots107, the combined frequency of four alleles (81, 109, 113, 117) was greater than 0.95 in all stocks, but stock differentiation was nonetheless apparent. For

example, the frequency of Ots107⁸¹ was 0.135 in Great Central Lake sockeye salmon, but < 0.010 in the other two stocks (Table 3). Variation in allelic frequencies at Ots108 was evident among stocks, with the frequency of Ots108¹²² ranging from 0.000 to 0.196. Strong genetic differentiation among these three stocks was evident at all six microsatellite loci.

Comparison of the relative magnitude of differentiation among stocks and among samples from the same stock collected in different years showed that differentiation among stocks always exceeded temporal variation within stocks and was on average 12 times greater (Fig. 2; Table 4). At Ots3, the differences among stocks were 235 times greater than the observed annual variability. With data combined over years, individual locus F_{ST} estimates ranged from 0.013 to 0.107, with an overall value of 0.056 (Table 4). The loci displaying the greatest differentiation among stocks were Ots3 and Omy77, whereas Ots103 displayed the least differentiation. Sproat Lake and Great Central Lake stocks, both in the same river drainage, were genetically the most similar (pairwise F_{ST} estimate over all loci: 0.032). The Great Central Lake and Henderson Lake stocks were more genetically distinct

Table 3

Observed allele frequencies at six microsatellite loci for three stocks of Barkley Sound sockeye salmon. Alleles have been designated by the lower size limit of the bin. *n* is the number of fish scored at each locus in each stock.

Allele	Sproat	Great Central	Henderson	Allele	Sproat	Great Central	Henderson
Omy7				Ots103			
<i>n</i>	264	303	305	<i>n</i>	221	304	308
90	0.000	0.000	0.002	144	0.007	0.021	0.000
92	0.011	0.003	0.002	152	0.043	0.026	0.000
94	0.449	0.172	0.005	156	0.027	0.002	0.002
96	0.042	0.020	0.000	160	0.009	0.018	0.003
98	0.006	0.007	0.000	164	0.034	0.008	0.013
100	0.125	0.231	0.277	168	0.016	0.015	0.006
102	0.011	0.015	0.030	172	0.038	0.033	0.019
104	0.216	0.361	0.546	176	0.020	0.025	0.034
106	0.002	0.002	0.020	180	0.029	0.064	0.080
108	0.040	0.005	0.000	184	0.032	0.076	0.057
110	0.051	0.054	0.100	188	0.050	0.178	0.073
112	0.002	0.003	0.002	192	0.305	0.268	0.312
114	0.004	0.086	0.008	196	0.269	0.151	0.185
116	0.038	0.040	0.010	200	0.075	0.079	0.130
118	0.004	0.002	0.000	204	0.043	0.028	0.071
Ots3				Ots107			
<i>n</i>	219	296	303	<i>n</i>	269	307	310
74	0.112	0.127	0.086	81	0.006	0.135	0.000
78	0.000	0.000	0.003	101	0.000	0.002	0.000
80	0.000	0.000	0.002	105	0.000	0.000	0.005
82	0.000	0.003	0.005	109	0.084	0.062	0.053
84	0.002	0.000	0.000	113	0.866	0.762	0.913
86	0.000	0.002	0.000	117	0.030	0.036	0.029
88	0.543	0.378	0.114	121	0.015	0.003	0.000
92	0.002	0.007	0.000	Ots108			
93	0.274	0.394	0.526	<i>n</i>	214	199	269
96	0.000	0.002	0.005	122	0.196	0.106	0.000
97	0.007	0.005	0.013	126	0.014	0.035	0.004
99	0.059	0.073	0.243	130	0.002	0.000	0.000
103	0.000	0.005	0.000	133	0.002	0.000	0.000
105	0.000	0.005	0.003	137	0.000	0.128	0.002
Ots100				Ots108			
<i>n</i>	242	321	282	141	0.002	0.008	0.002
130	0.010	0.003	0.000	145	0.007	0.010	0.000
134	0.039	0.006	0.000	149	0.121	0.038	0.035
138	0.000	0.002	0.000	153	0.056	0.098	0.007
142	0.006	0.000	0.002	156	0.136	0.095	0.178
150	0.008	0.002	0.004	160	0.086	0.146	0.229
154	0.025	0.037	0.025	164	0.189	0.163	0.158
158	0.256	0.364	0.504	168	0.042	0.070	0.048
162	0.169	0.064	0.133	172	0.035	0.038	0.043
166	0.052	0.047	0.064	177	0.068	0.050	0.216
170	0.002	0.003	0.007	182	0.016	0.010	0.039
174	0.002	0.006	0.004	187	0.019	0.005	0.035
179	0.324	0.202	0.193	192	0.005	0.000	0.004
184	0.085	0.115	0.050	197	0.002	0.000	0.000
190	0.008	0.107	0.007				
195	0.012	0.039	0.009				
200	0.000	0.002	0.000				

(pairwise F_{ST} estimate: 0.042), and the Sproat Lake and Henderson Lake stocks showed the greatest differentiation (pairwise F_{ST} estimate: 0.091).

Estimation of stock composition

The three loci with the highest F_{ST} estimates (Omy77, Ots3, and Ots107) also possessed the highest ratio of

spatial to temporal variation (Table 4) and were therefore selected to form the core database for the analysis of the simulated mixtures. The number of loci used in the determination of stock composition or mixture sample size had little effect upon the accuracy of the estimated stock compositions (Table 5). Precision of the estimated stock compositions increased as both the number of loci and mixture size used in the determination increased. However, different options were available to obtain estimates of a desired precision. For example, higher levels of precision were obtained with four loci (Omy77, Ots3, Ots107, Ots100) in conjunction with a 150-fish sample size (600 units of data) than with all six loci and a 100-fish sample (600 units of data) (Table 5). The coefficient of variation for the estimated proportion of the predominant Great Central Lake stock was always less than that for estimated proportions of the other two stocks in the mixture. For the 4 loci in 150-fish mixture analysis, the coefficient of variation for the estimated proportion of Great Central Lake fish was 12%, whereas it was 22% for Sproat Lake fish, and 38% for Henderson Lake fish. The simulations indicated that fewer than six microsatellite loci could be used to provide reasonably precise data and accurate estimates of sock-

eye salmon stock composition for Barkley Sound fishery samples.

Conditional maximum likelihood estimation can overestimate the relative abundance of rare stocks. For Barkley Sound, this would likely be the Henderson Lake stock. The precision of data and accuracy of stock compositions estimates were investigated for mixture samples of 100 fish composed of 2% Henderson Lake (38% Sproat Lake, 60% Great Central Lake) and 5% Henderson Lake (35% Sproat Lake, 60% Great Central), where stock compositions were estimated by using the four microsatellite loci (Omy77, Ots3, Ots100, and Ots107) generally used for estimation of stock compositions in the 1997 fishery samples. Estimated stock compositions of the simulated 100 mixtures for the 2% Henderson Lake composition were 2.5% (SD=3.1%) Henderson Lake, 34.3% (SD=9.3%) Sproat Lake, and 60.4% (SD=9.3%) Great Central Lake. For the 5% Hender-

Table 4

F_{ST} estimates and the ratio of the variance components attributable to among and within stock differentiation (over time) for six microsatellite loci of Barkley Sound sockeye salmon. Standard deviation of F_{ST} estimates is given in parentheses.

Locus	F_{ST}	Variance ratio
Omy77	0.107 (0.094)	20.3
Ots3	0.099 (0.089)	235.2
Ots100	0.027 (0.013)	5.8
Ots103	0.013 (0.007)	3.9
Ots107	0.043 (0.035)	6.3
Ots108	0.039 (0.018)	3.6
All	0.056 (0.032)	11.8

Table 5

Average estimated stock composition (%) of simulated mixtures of Barkley Sound sockeye salmon based on variation at three to six microsatellite loci. True mixture percentages were as follows: Sproat Lake 30%, Great Central Lake 60%, and Henderson Lake 10%. The three loci initially used to estimate the percentages of the mixtures were Omy77, Ots3, and Ots107, and Ots100, Ots108, and Ots103 were added sequentially. Each mixture was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each baseline stock with replacement to obtain a new distribution of allele frequencies, with the same sample size in the new distribution as in the original one. Standard deviation is given in parentheses.

Source and loci	Mixture size (no. of fish)			
	100	150	200	300
Sproat Lake				
3	30.7 (9.98)	31.1 (8.51)	30.8 (6.81)	30.7 (6.48)
4	30.7 (8.21)	30.8 (6.87)	30.8 (6.38)	30.2 (4.44)
5	30.1 (7.54)	30.6 (5.87)	30.0 (4.44)	29.4 (4.18)
6	31.2 (7.23)	29.0 (5.27)	29.8 (4.42)	29.8 (4.00)
Great Central Lake				
3	60.5 (11.74)	60.1 (9.27)	60.2 (7.40)	59.7 (7.74)
4	60.4 (9.41)	59.8 (7.40)	59.7 (6.88)	60.7 (5.21)
5	60.1 (8.26)	60.0 (6.63)	60.1 (5.11)	60.8 (4.50)
6	58.8 (8.03)	60.9 (5.96)	60.1 (5.09)	59.9 (4.31)
Henderson Lake				
3	8.8 (5.62)	8.8 (4.84)	9.0 (3.89)	9.6 (3.98)
4	8.8 (5.30)	9.3 (3.53)	9.6 (3.52)	9.1 (2.89)
5	9.8 (4.56)	9.5 (3.73)	9.9 (2.76)	9.8 (2.52)
6	10.0 (4.33)	10.1 (3.70)	10.0 (2.81)	10.3 (2.39)

son Lake composition, estimated stock compositions were 5.3% (SD=3.9%) Henderson Lake, 34.3% (SD=8.2%) Sproat Lake, and 60.4% (SD=8.9%) Great Central Lake. No significant bias was observed when Henderson Lake sockeye salmon composed 5% or less of the mixture.

Application of estimates to 1997 fisheries

Although estimated stock contributions varied according to sampling period, sockeye salmon from Great Central Lake tended to predominate in all fisheries at any week (Table 6). However, differences in stock composition estimates among fishing gears were evident. In the commercial gillnet fishery, Great Central Lake sockeye salmon constituted about 70% of the catch (Table 6). In the gillnet test fishery, the proportion of Great Central Lake sockeye generally varied between 55 and 75% prior to July 25th. In the purse-seine test fishery, they accounted for about 50–55% of the catch. Higher proportions of Great Central Lake sockeye salmon were observed in the selective gillnet gear than in the more nonselective purse-seine gear. For example, for the week ending 4 July, Great Central Lake sockeye were estimated to have represented 70–75% of the catch in the commercial gillnet fishery and in the gillnet test fishery, but only about 40% of the catch in the seine test fishery. Although the samples analyzed from the purse-seine fishery were derived from more inland locations than those from the commercial and test gillnet fisheries, the differences in proportions of Great Central sockeye salmon more likely resulted from differences in gear selectivity than from differences in stock distribution because fish from all three stocks are generally distributed throughout Barkley Sound and Alberni Inlet when present.

Sockeye salmon stock from Henderson Lake are the smallest salmon exploited in the fishery, and thus the most vulnerable to overfishing in the mixed-stock harvest that takes place. Henderson Lake fish, which do not have to travel through Alberni Inlet in their spawning migration, were apparently caught in fisheries throughout Alberni Inlet, although there was a high degree of uncertainty about whether they were caught in the aboriginal fishery at the extreme head of Alberni

Table 6

Estimated stock compositions (%) for sockeye salmon from three lakes sampled in gillnet test fisheries, seine test fisheries, commercial fishery openings, a native fishery, and recreational fishery in Barkley Sound during 1997. Four loci (Ots3, Ots100, Ots107, and Omy77) were used to estimate stock composition. *n* is the number of fish analyzed, and standard deviation of the estimates is given in parentheses.

Source	Week ending	<i>n</i>	Sproat	Great Central	Henderson
Commercial	4 Jul	118	25.8 (7.3)	73.9 (8.2)	0.3 (2.8)
Commercial ¹	11 Jul	95	22.3 (6.2)	71.1 (7.9)	6.2 (3.8)
Commercial ¹	18 Jul	95	29.7 (6.9)	63.0 (7.8)	7.3 (4.8)
Seine	27 Jun	120	35.9 (7.3)	56.6 (8.0)	7.5 (3.4)
Seine	4 Jul	111	48.4 (8.6)	42.2 (10.4)	9.0 (5.4)
Seine	18 Jul	117	37.3 (7.0)	59.6 (7.8)	3.1 (4.6)
Gillnet	20 Jun	50	34.4 (10.3)	65.6 (10.4)	0.0 (0.9)
Gillnet	27 Jun	50	36.2 (10.9)	51.0 (12.9)	12.9 (6.8)
Gillnet	4 Jul	50	21.2 (9.2)	71.9 (10.5)	6.7 (5.1)
Gillnet	11 Jul	50	33.1 (9.7)	54.1 (11.7)	12.8 (7.7)
Gillnet	18 Jul	50	24.5 (11.8)	72.2 (13.6)	3.4 (6.3)
Gillnet	25 Jul	50	19.9 (11.5)	60.0 (13.8)	20.2 (9.1)
Gillnet	1 Aug	50	28.2 (11.6)	42.7 (13.7)	29.1 (10.3)
Aboriginal	18 Jul	86	45.0 (8.4)	52.8 (8.5)	2.2 (2.3)
Recreational	18 Jul	33	33.5 (12.3)	54.8 (13.3)	11.7 (8.4)

¹ Additional loci, Ots103 and Ots108, were used in estimation of stock composition.

Inlet (Table 6). Henderson Lake sockeye salmon generally represent 10% or less of the catch, except for sockeye salmon sampled after 18 July in the gillnet test fishery, when the relative abundance of Henderson Lake sockeye salmon substantially increased. By late July, Henderson Lake sockeye salmon constituted nearly 30% of the gillnet test fishery sample.

Discussion

DNA variation at microsatellite loci is becoming an increasingly important tool in fisheries research and management (see review by O'Connell and Wright [1997]). In salmonids, microsatellite loci are generally characterized by high levels of variability and differentiation among spawning populations (Angers et al., 1995; McConnell et al., 1997; Seeb et al., 1998), even in very localized areas (Beacham and Dempson, 1998). The feasibility of applying biological markers to salmon stock identification is enhanced when they display limited annual variation. With temporal stability of the discriminating characters, annual surveys of contributing populations are unnecessary once they have been adequately characterized. As for other neutral genetic markers (Wood et al., 1994; Beacham et al., 1996), temporal stability of allele frequencies at microsatellite loci has generally been observed in salmonid populations (Small et al., 1998). For popula-

tions in which annual variation has been detected, the magnitude of variation has been substantially less than that among populations (Nielsen et al., 1997; Beacham and Wood, 1999).

For sockeye salmon, in which the greatest geographic determinant of neutral genetic differentiation is the nursery lake (Wood, 1995), the task of identifying the contributions of three different lake systems to a mixed-stock sample should be relatively straightforward. Although significant genetic variation can occur among spawning sockeye salmon subpopulations isolated by time or space (or both) within a lake system, the extent of this variation is consistently much less than that observed among lakes—even those lakes within a single drainage system (Wood, 1995). Each of the three lakes is the confluence of multiple tributaries and may harbor genetically differentiated subpopulations of sockeye salmon. The spawning ground samples in our study were collected from locations within each lake system at which fish from more than one subpopulation may have been present, and different subpopulations may have been sampled among years. Thus, the departure of Omy77 (and Ots108 for Henderson Lake) genotypes from Hardy-Weinberg equilibrium and significant annual variation observed at these loci might both have reflected subpopulation differentiation in allele frequencies. It is unlikely that the heterozygote deficiency observed at Omy77 in Sproat Lake and Henderson Lake sockeye salmon would be a result of a null allele because genotypic frequencies of other sockeye salmon stocks surveyed at this locus have been in Hardy-Weinberg equilibrium (Beacham and Wood, 1999). Nevertheless, the level of differentiation at Omy77 was about 20 times greater among lakes than was the temporal variation observed within lakes. For all six microsatellite loci surveyed, differences among lakes were on average 12 times greater than variation within populations, confirming the relative stability of the microsatellite loci in Barkley Sound sockeye salmon populations over the 5–8 yr sampling period.

The six microsatellite loci used in the current study were also surveyed in nine sockeye salmon stocks of the Nass River drainage in northern British Columbia (Beacham and Wood, 1999). In the Nass River, the three loci displaying the greatest differentiation among stocks were Ots100 ($F_{ST}=0.131$), Ots3 ($F_{ST}=0.111$), and Ots108 ($F_{ST}=0.084$), whereas in the Barkley Sound stocks, the three most discriminating loci were Omy77 ($F_{ST}=0.107$), Ots3 ($F_{ST}=0.099$), and Ots107 ($F_{ST}=0.043$). The fact that loci differed in their relative levels of variation between the two areas is not surprising given the rapid evolution of microsatellite loci and the likelihood that the regions were founded postglacially by different sockeye salmon “races” (Wood, 1995). For stock identification applica-

tions, surveys of microsatellite variation in each geographic region of interest will generally be necessary to determine which loci are the most effective in differentiating local populations.

Effective assessment and management of sockeye salmon production in Barkley Sound is dependent upon determination of stock composition in fishery catches. Previous evaluation has indicated that the application of microsatellite technology to stock identification can provide the most reliable and cost-effective results (Beacham et al., 1998), but determination of the feasibility of such technology for Barkley Sound fisheries awaited examination of the relation between the number of loci used, the sample size of the stock mixture to be analyzed, and the precision of the estimated stock contributions. For any stock identification application, the optimal combination of number of loci surveyed and number of fish sampled from the catch is dependent on the genetic distance among stocks, the desired precision for an individual stock estimate, and the cost of the analysis for each locus.

The simulated mixtures evaluated for Barkley Sound sockeye salmon indicated that microsatellite variation could be used to provide accurate and reasonably precise estimates of individual stocks in the catch mixtures. They further indicated that although genotypic frequencies at Omy77 and Ots108 were not in Hardy-Weinberg equilibrium in some stocks, but assumed to be so in the stock composition estimation procedure, the violation of this assumption did not have a marked influence on the accuracy of the estimated stock compositions. The precision, but not accuracy, of the estimated contributions increased with both the number of loci (from 3 to 6) and the sample size of the mixture (from 100 to 300). For sample sizes of 150 fish and larger, a greater increase in precision for stock contribution estimates could always be achieved by increasing the number of loci surveyed to six than by increasing the sample size to 300. However, these simulations did not include estimation of the random error associated with sampling only a portion of the catch, and this error will always be reduced by increasing sample size. The level of precision of an estimated stock contribution increased with the contribution of the stock to the mixture. For estimation of the more abundant Great Central and Sproat sockeye salmon, the increase in precision afforded by additional data was approximately equivalent whether more fish (beyond 150) or more loci were analyzed (i.e. approximately equally precise stock contribution estimates were achieved by analyzing four loci in 300 fish and six loci in 200 fish). However, estimation of the small (10%) Henderson Lake contribution to the mixture was more sensitive to sample size and was more precise in the analysis of four loci in 200 fish than of six loci in 150 fish.

Successful application of microsatellite loci to estimation of stock composition in mixed-stock fisheries requires that loci be chosen that highlight differences among stocks to be separated and that adequate numbers of fish in the baseline stocks be surveyed to provide reliable estimates of allele frequencies, and thus genotypic frequencies used in the conditional maximum likelihood analysis. Microsatellite loci can contain a large number of alleles, and baseline sample sizes need to be of sufficient size to ensure that alleles present in fish from a stock in the mixture have also been observed in the baseline samples. Binning low-frequency similar-size alleles (Small et al., 1998) is also a strategy to consider in practical applications.

Although simulated mixtures can provide insights into the expected performance of the mixture analysis, the stock contribution estimates for actual fishery samples can only be evaluated by corroboration with data from other sources. Two supportive sources of independent information occur: time of return of the Henderson Lake stock and the typical catch composition for Barkley Sound that was previously derived from parasites. In Barkley Sound, the time of return of Henderson Lake sockeye salmon has been reported to be later than that of either Sproat Lake or Great Central Lake fish (Steer et al., 1988). For example, in 1984, Henderson Lake sockeye salmon were evident, on the basis of parasite analysis, in the commercial fishery prior to 27 June but increased in relative abundance after that time. The current analysis indicated that Henderson Lake sockeye salmon were absent from, or at low abundance in, the 1997 commercial fishery prior to the week of 4 July. Analysis of the gillnet test fishery and purse-seine samples indicated that the proportion of Henderson Lake sockeye salmon in those catches was low until mid-July but thereafter was substantial, consistent with a later time of arrival of the Henderson stock in Barkley Sound. In a typical return year, about 60% of the Barkley Sound sockeye salmon catch is derived from Great Central Lake, 30% from Sproat Lake, and 10% from Henderson Lake (Steer et al., 1988). Estimated stock compositions for the 1997 fishery catches are in reasonable agreement with the expected stock contributions. Results of the simulation analysis indicated that more precise, but not necessarily more accurate, estimates of the stock contributions (especially that from Henderson Lake) could have been obtained for the fishery catches if sample sizes had been larger than 50 (for the gillnet test fisheries) or approximately 100 (for the purse-seine test and commercial gillnet fisheries).

Differences in estimated stock composition were obtained for the purse-seine and gillnet test fisheries in July samples, where higher proportions of Great Central Lake sockeye salmon were observed in the

gillnet fishery samples. Although the fishery samples came from different areas (the purse-seine samples were collected farther inland in Alberni Inlet than were the gillnet samples), the most likely explanation of the difference in estimated proportions of stock composition between the two gears is a difference in size selectivity. Sockeye salmon caught in purse seines in Barkley Sound are generally more variable in size and of smaller mean size than those caught in gill nets (Steer et al., 1986). Probably gill nets were more selective for Great Central Lake sockeye salmon than for Sproat Lake salmon. Thus, it is important to estimate stock contributions to a fishery catch based on samples collected with the type of gear employed in the fishery. Furthermore, the analysis of catch samples to estimate the stock composition of fish present in an area (as opposed to those caught in an area) will be biased to the degree that the sampling gear nonrandomly catches the fish that are present. The results of this study and other analyses (Beacham et al., unpubl. data) indicate that for salmonids, different gear types sample the various stocks in a stock mixture with very different efficiencies.

Differentiation among local spawning populations that are relatively stable over time provides the basis for applying biological markers to problems of salmonid fisheries management. This study confirmed our expectation that the level of differentiation observed at microsatellite loci among sockeye salmon of the three major lake systems draining into Barkley Sound is sufficient and stable to assess stock composition of the fishery catches. The abundance of highly polymorphic microsatellite loci in salmonid fish, the relative ease of nonlethal sample collection, and the moderate cost per fish for laboratory analysis combine to provide a technology that will become increasingly used in the assessment and management of salmonid fisheries.

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